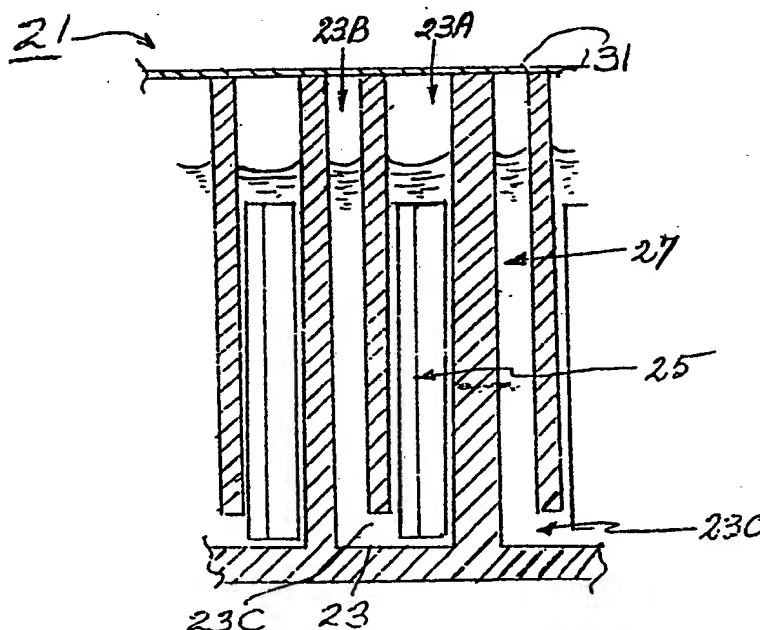


## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| <b>(51) International Patent Classification <sup>5</sup> :</b><br><b>G01N 21/03, 31/20, 33/53</b><br><b>G01N 33/543, 33/545, 33/552</b>  | <b>A1</b> | <b>(11) International Publication Number:</b> <b>WO 91/18276</b><br><br><b>(43) International Publication Date:</b> 28 November 1991 (28.11.91)  |
| <b>(21) International Application Number:</b> PCT/US91/03589<br><b>(22) International Filing Date:</b> 21 May 1991 (21.05.91)<br><br><b>(30) Priority data:</b><br>527,069                      22 May 1990 (22.05.90)                      US<br><br><b>(71)(72) Applicants and Inventors:</b> KELLY, Kenneth, A. [CA/US]; CARD, Benjamin, D. [CA/US]; P.O. Box 202003, Arlington, TX 76006 (US). RECINELLI, Nevio [IT/IT]; COSIMI, Simonetta [IT/IT]; Bioallergy, Srl., Via Monte Solarolo, 87, I-00054 Fiumicino (IT).<br><br><b>(74) Agent:</b> ZOBAL, Arthur, F.; Wofford, Fails, Zobal & Mantooth, 110 West Seventh Suite 500, Fort Worth, TX 76102-7077 (US). |           | <b>(81) Designated States:</b> AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES, ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KP, KR, LU (European patent), NL (European patent), SE (European patent).<br><br><b>Published</b><br><i>With international search report.</i> |

**(54) Title:** MICROASSAY STRIP DEVICE FOR IMMUNOASSAY**(57) Abstract**

A multichamber microassay strip device (21) is described to be used for the detection of analytes in biological fluids by immunoassay. The chambers (23) of the strip (21) are U-shaped and may be coated with immunochemical reagents or may contain solid phase segments (25) coated with immunochemical reagents. The microassay strip (21) is designed particularly for use in an automated immunoassay system.

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MICROASSAY STRIP DEVICE FOR IMMUNOASSAY <sup>Background of the Invention</sup>

Analytes, such as antibodies or antigens, in biological fluids, such as serum, are generally detected by specific immunoassay reaction of an antibody with antigen to form an immune complex. The reagent, antibody or antigen, which reacts specifically with the analyte is labelled to permit measurement and quantitation of the analyte. The labels may be radioactive emitters such as Iodine-125, or enzymes such as urease or alkaline phosphatase which on reaction with substrate produce a color or fluorescence change which can be measured optically.

Solid phase immunoassays utilize a solid support, such as paper discs, plastic test tubes, cellulose threads, microtiter plastic wells, to which one of the reactants in the immunoassay is attached. Solid phase immunoassays facilitate the separation of the bound phase of the reaction, which is the immune complex formed on the surface of the solid phases, from the free phase of the reaction which is the reagent(s) in the surrounding fluid.

Many types of solid phases have been developed to facilitate performing immunoassays. Reagents such as allergens have been coupled covalently to paper discs or beads which can be placed in test tubes to quantitate IgE antibodies in the serum of patients by the RadioAllergoSorbent Test or RAST (Bennich, H.H., Johansson, S.G.O. and Wide, L.E. 1973, U.S. Patent 3,720,760). These discs or beads, however, could easily be removed inadvertently from their tubes during an assay. Plastic tubes, 12 x 75mm, made of polystyrene or polyvinyl chloride, have been coated on their inner surface with reactant to overcome this problem (Ling, C. 1975, U.S. Patent 3,867,517; Catt, K.J., 1972, U.S. Patent 3,646,346). Coated tubes are bulky reagents

from a commercial production point of view and do not lend themselves readily to automation. The most significant improvement in automated solid phase immunoassay is the use of microtiter wells, made of polystyrene or polyvinyl chloride and usually contain 0.5 milliliters of reagent. These wells can be moulded into strips of 8 to 12 wells or plates of 8 x 12 wells (Voller, A., Bidwell, D.E., Hultdt, G. and Bartlett, A., 1974, Bull. WHO, 51, 209-221). Instrumentation has been developed for automation of immunoassays carried out in microtiter wells to dispense reagents into the wells, to wash the wells to remove free reactants and to measure the end product of the immunoassay reaction, such as a color change in substrate reagent in the well.

The microtiter well, although it offers several advantages over coated tubes with regard to automation, still requires a significantly large volume of reagent, 50 to 200 microliters. A capillary device for multiple analytes has been developed by K.A. Kelly (PCT Patent Application WO 89/00290) in which polyvinyl chloride solid rod-like segments, 10mm long by 1.8mm diameter, are coated with allergens and inserted in a capillary tube of 2.3mm inner diameter. A syringe is used to fill and empty the capillary device of reagents. This device has been applied to the detection of IgE antibodies to multiple allergens in an enzyme immunoassay. The advantages of this invention in solid phase immunoassays are that the volume of reagents required in the capillary format is only 20 microliters per segment, there is a much larger surface area to volume ratio than the other microtiter well or coated tube solid phase assays so that the reaction proceeds much quicker to equilibrium. In addition, the enzyme-catalyzed color change within the capillary tube can be measured directly through the wall of the tube.

## Summary of the Invention

The present invention describes a device, the "ENEAs" microassay strip, which can be used to carry out a solid phase immunoassay for analytes in a fluid. The device consists of a rectangular strip containing a plurality of vertical U-shaped reaction wells of capillary dimensions. In the larger capillary chamber of each reaction well is inserted a rod-like segment, to which surface has been attached a reagent which reacts specifically with the analyte in the fluid. The strip may contain twenty-five of these capillary wells. The strip is designed for use in an automated enzyme immunoassay: The analyte fluid is dispensed into the chamber containing the reactive segment and allowed to react forming an insolubilized immune complex on the segment surface. The free analyte in solution is removed by addition of wash fluid into the reaction arm of the U-chamber and aspiration from the other arm of smaller diameter. After a subsequent and similar reaction step with an antibody-enzyme conjugate and washing, substrate is added. The amount of analyte on the surface of the segment is estimated by measurement of the color change in the reaction chamber spectrophotometrically through the wall of the chamber.

## Description of the Drawings

Figure 1 is a sectional view through the microassay strip device 21 showing:

- (a) top view
- (b) side view
- (c) bottom view of the device

Figure 2 is a schematic representation of a coordinate workstation with microassay strips in place in an automated immunoassay.

Figure 3 is an enlarged portion of the microassay strip device showing the U-shaped chamber 23 and reactive segment 25.

## Detailed Description

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An example of the "ENEAs" microassay strip 21 in accordance with the present invention is illustrated in Fig. 1. The strip is basically a rectangular piece with a length of 145mm, height 16mm and thickness 3mm. It contains twenty-five U-shaped reaction chambers 23; however, the actual number of chambers may vary. Each U-shaped chamber (Fig. 3) consists of one vertical arm 23A of inner diameter of 2.1mm and the other vertical arm 23B of inner diameter of 1.2mm. The two vertical arms are connected by a horizontal base arm 23C of 1.2mm <sup>diameter</sup>. The pitch or distance of separation of one vertical arm of a chamber from the similar arm of the adjacent chamber is 4.71mm. The above dimensions of the microassay strip and U-shaped chambers are preferred dimensions though not essential to the present invention. The microassay strip may be made from transparent or optically clear glass or plastic material such as polystyrene or polyvinyl chloride or other optically clear polymeric materials.

The capillary U-shaped reaction chamber as described permits reagent fluid to be added to or removed from either arm of the chamber. The larger bore chamber is designed to contain one rod-like plastic segment 25, 10mm height by 1.8mm diameter, with an immunoassay reagent affixed to the surface of the segment. The volume of reagent required to fill the U-shaped reaction chamber as described is less than 50 microliters.

Although the "ENEAs" microassay strip in accordance with the present invention has wide application to the detection of analytes in a fluid, it is especially well suited for solid phase immunoassays. The following detailed description will be limited to immunoassay reactions with it being understood that the present invention has wide application to detection systems other than antibody-antigen reactions.

In a preferred embodiment, the "ENEAs" microassay strip is used for the detection of antibodies of the IgE class in the serum or plasma of allergic patients by a conventional enzyme immunoassay.

Allergenic reagents such as aqueous extracts from grass, weed or tree pollens, from animal dander or from various food substances are attached to the surface of the rod-like segments. Exemplary materials of the solid phase segments include glass or plastic polymers such as polystyrene, polyvinyl chloride, etc. The allergenic reagents may be attached to the solid phase surface by known techniques, such as passive adsorption or covalent bonding. Exemplary procedures of covalent bonding involve treating the polymer surface with glutaraldehyde prior to reaction with the allergen in a 2-step procedure, or reacting mixture of allergen and glutaraldehyde with the polymer surface in a 1-step procedure (Parsons, G.H., 1978, U.S. Patent 4,069,352). The allergen solid phase is subsequently treated with an inert substance, such as albumin, to saturate residual active sites on the surface and to stabilize the coated surface for long term use. The allergen coated segment is next placed in the larger bore chamber for immunoassay.

In the conventional assay, fluid serum sample (40-50ul) 27 is dispensed into the chamber. The reaction is allowed to proceed for a sufficient time at a suitable temperature to allow first antibodies in the serum to complex specifically with allergens on the segment surface. Typical conditions of incubation are 60 minutes at ambient room temperature (18-30C). The chamber and segment within are washed repeatedly to remove free fluid reactants.

A suitable enzyme-second antibody reagent, such as Urease coupled covalently to antibody specific for human IgE, is next added and incubated under appropriate conditions to allow the enzyme-second antibody to form immune complexes with the IgE first antibody on the segment surface. The chamber and segment within are again washed repeatedly to remove free enzyme-antibody reactants. A substrate-indicator system for Urease enzyme, such as a urea-bromocresolpurple pH indicator solution mixture, is next added to the chamber. In this detection system a positive yellow to purple color change will occur which can be measured by placing the microassay strip in a suitable spectrophotometer.

One modification of the "ENEAs" microassay strip 21 involves attaching the first immunochemical reagent of antibody or antigen directly to the inner wall of the U-shaped capillary chamber, rather than to a reactive segment which can be placed within the capillary chamber. Microassay strips prepared by this modification may be prepared with attached immunochemical reagents as described for reactive segments and may be used in an immunoassay in a similar manner to microassay strips containing reactive segments within the U-shaped capillary chambers.

The "ENEAs" microassay strip may be used for a variety of solid phase immunoassays wherein one of the antibody or antigen reactants is attached to the surface of the solid phase to insolubilize by immune complexing with the other reactant in the fluid phase. The labels in the detection system may include any combination of enzyme/substrate systems, or antibody or antigen reactants labelled with fluorescent



markers such as fluorescein, with appropriate optical instrumentation for measurement.

The "ENEAS" microassay strip is particularly suited for an automated immunoassay system. In an embodiment of such an automated system depicted generally in Fig. 2, a large number of these immunoassay strips are fixed on a movable platform. The system can be programmed to perform a multitude of steps in sequence. These steps include addition of a number of samples of unknown analyte concentrations, standards and controls samples to appropriate reaction U-chambers, washing after a predetermined incubation time, addition of labelled second reactant, washing after a predetermined incubation time, followed by measurement and printout of results. The advantage of this system is that it is fully automated after loading of reagents and programming.

The unique features of a capillary U-shaped reaction chamber are that fluid reagent volumes are minimal ( $\leq 50\mu\text{l}$ ), there is a large surface area to volume ratio which maximizes the rate of immunochemical reactions to equilibrium, and proper washing of the solid phase after each reaction step can be carried out more easily and thoroughly. Wash solution reagent is dispensed into one arm of the U-shaped chamber 23 and removed by suction applied at the other arm of the chamber. This provides a unidirectional flow through the chamber which more efficiently removes fluid reactants in the chamber. Instrumentation to dispense and aspirate wash solution for the U-shaped chamber are available and may be easily incorporated in the automated system (Fig. 2).

Specific applications of the "ENEAs" microassay strip in the field of allergen detection and management include the following examples. Strips can be prepackaged in which all the solid phase segments per strip are coated with the same allergen reactant. A number of analyte serum samples and standards may be added to the strip to quantitate the level of antibody in the samples to that particular allergen. Another application involves assembling segments coated with difference allergens in the single microassay strip and adding a single analyte sample to each well of the strip to quantitate IgE antibodies to a panel of allergens. A third application involves the use of segments coated with antibody to human IgE in the strip so that samples can be assayed for their level of IgE immunoglobulin.

In production of the microassay strip, reactive segments 25 may be inserted into the U-shaped chambers 23, stabilizing liquid added if necessary, and the upper surface of the microassay strip 21 sealed with <sup>removable</sup> tape to retain the contents of the U-chambers until use in assay.

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What is claimed is:

1. A microassay strip device for the detection and quantitation of analytes in a biological fluid sample by immunoassay comprising:

- (a) a supporting block made from optically clear material containing a number of vertical U-shaped capillary chambers hollowed out from the block, and
- (b) immunochemically reactive water-insoluble segments located in one vertical arm of each of said U-shaped chambers.

2. The microassay strip device according to claim 1 wherein said strip device is made from optically clear material, such as glass or polymeric plastic materials or derivatives thereof.

3. The device of claim 1 wherein said water-insoluble segments are of glass or polymeric plastic materials or derivatives thereof.

4. The device of claim 1 wherein said immunochemically reactive water-insoluble segments have antibody or antigen reagents attached to the surface of said segments by passive or covalent bonding.

5. The device of claim 1 wherein all said reactive segments contained within said strip device have the same immunochemical reagent attached to their surface.

6. The device of claim 1 wherein each of said reactive segments has a different immunochemical reagent attached to its surface.

7. A method for the detection and quantitation of analytes in a biological fluid sample by immunoassay with the use of a microassay strip device comprising a supporting block made from optically clear material containing a number of vertical U-shaped capillary chambers with a reactive water-insoluble segment located in each chamber, each segment having attached to its surface a first immunochemical reagent that reacts specifically with said sample analyte, the method comprising the steps of:

- (a) filling said capillary chamber with the fluid sample,
- (b) allowing the analytes in said fluid sample to react with said specific immunochemical reagent on said reactive segment,
- (c) removing said fluid sample from said capillary chamber,
- (d) washing said capillary chamber internally to remove sample components not specifically bound to reactive segment,
- (e) filling said capillary chamber with a labelled second immunochemical reagent capable of reacting with one of the reactants in step (b), namely the analyte in said fluid sample or the first immunochemical reagent on the surface of said reactive segment,
- (f) allowing the reaction involving the said labelled second immunochemical reagent to take place,
- (g) removing said labelled second immunochemical reagent from said capillary chamber,
- (h) washing said capillary chamber internally to remove said labelled second immunochemical reagent not specifically bound to said reactive segment,
- (i) measuring the amount of said labelled second immunochemical reagent bound specifically to the reactive segment.

8. The method of claim 7 wherein said second immunochemical reagent is labelled with a fluorescent molecule or luminescent molecule and the amount of said labelled second immunochemical reagent bound specifically to the reactive surface can be measured by measuring the fluorescence or luminescence emitted through the wall of the capillary chamber.

9. The method of claim 7 wherein said second immunochemical reagent is labelled with an enzyme molecule and the amount of said labelled second immunochemical reagent bound specifically to the reactive surface can be measured by addition of substrate reagent to the capillary chamber and measuring the amount of product formed by enzyme catalysis.

10. A microassay strip device for the detection and quantitation of analytes in a biological fluid sample by immunoassay comprising a supporting block made from optically clear material containing a number of vertical U-shaped capillary chambers within the block, with first immunochemical reagents attached to the surface of said U-shaped chambers.

11. The microassay strip device according to claim 1 wherein said strip device is made from optically clear material, such as glass or polymeric plastic materials or derivatives thereof.

12. The device of claim 10 wherein said first immunochemical reagent may be antibodies or antigens attached to the surface of said U-shaped chambers by passive or covalent bonding.

13. The device of claim 10 wherein all of said U-shaped chambers in a single microassay strip have the same immunochemical reagent attached to their surface.

14. The device of claim wherein said U-shaped chambers in a single microassay strip have different immunochemical reagents attached to their surface.

15. A method for the detection and quantitation of analytes in a biological fluid sample by immunoassay with the use of a microassay strip device comprising a supporting block made from optically clear material containing a number of vertical U-shaped capillary chambers

within the block with first immunochemical reagent attached to the surface of said U-shaped chambers, comprising the steps of:

- (a) filling said capillary chamber with the fluid sample,
- (b) allowing the analytes in said fluid sample to react with said first immunochemical reagent on the inner surface of said capillary chambers,
- (c) removing said fluid sample from said capillary chamber,
- (d) washing said capillary chamber internally to remove sample components not specifically bound to its surface,
- (e) filling said capillary chamber with a labelled second immunochemical reagent and allowing the reaction to take place,
- (f) removing said labelled second immunochemical reagent from the capillary chamber,
- (g) washing said capillary chamber to remove labelled second immunochemical reagent not specifically bound to the surface of the chamber, and
- (h) measuring the amount of said labelled second immunochemical reagent bound specifically to the reactive segment.

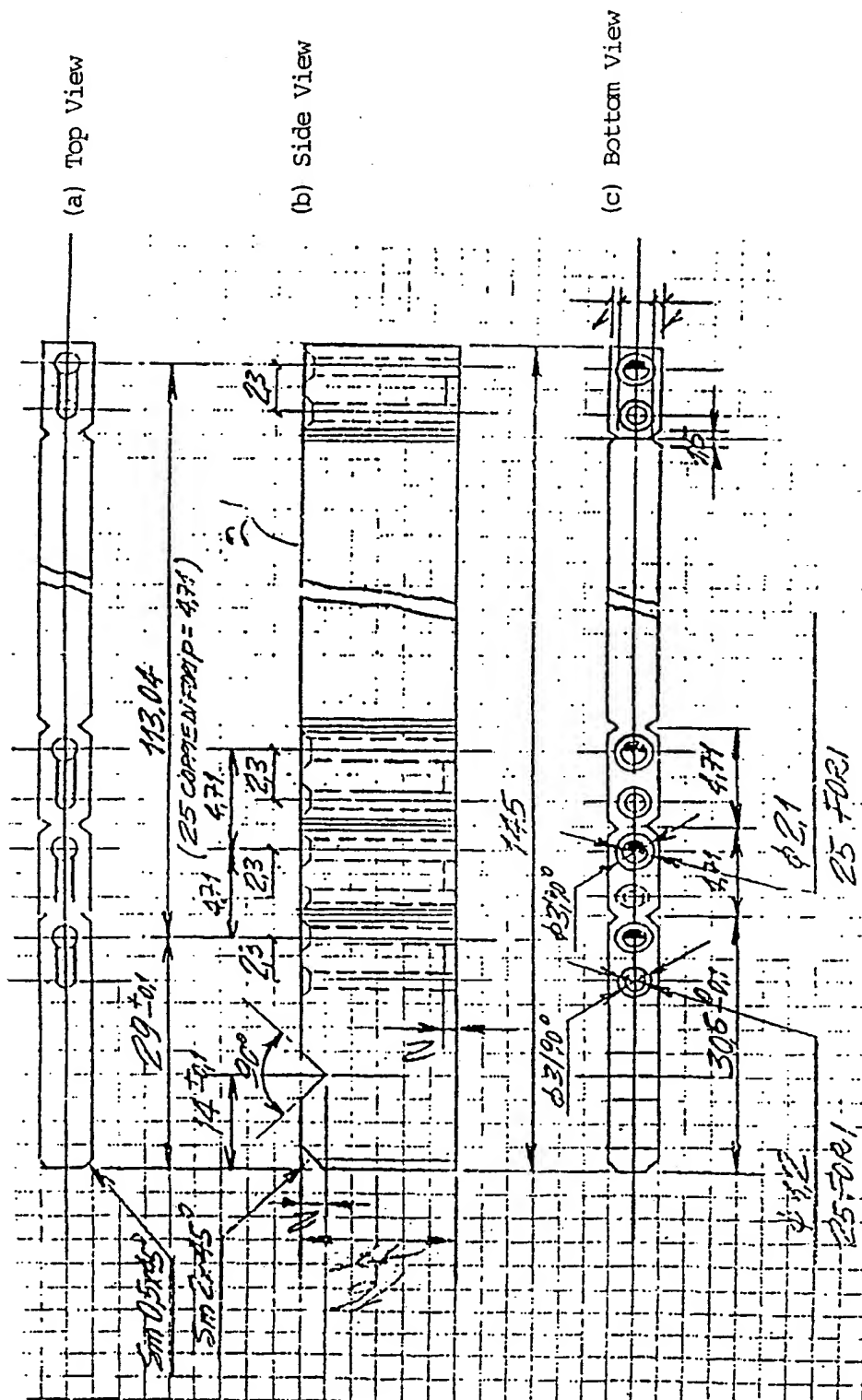


Fig. 1

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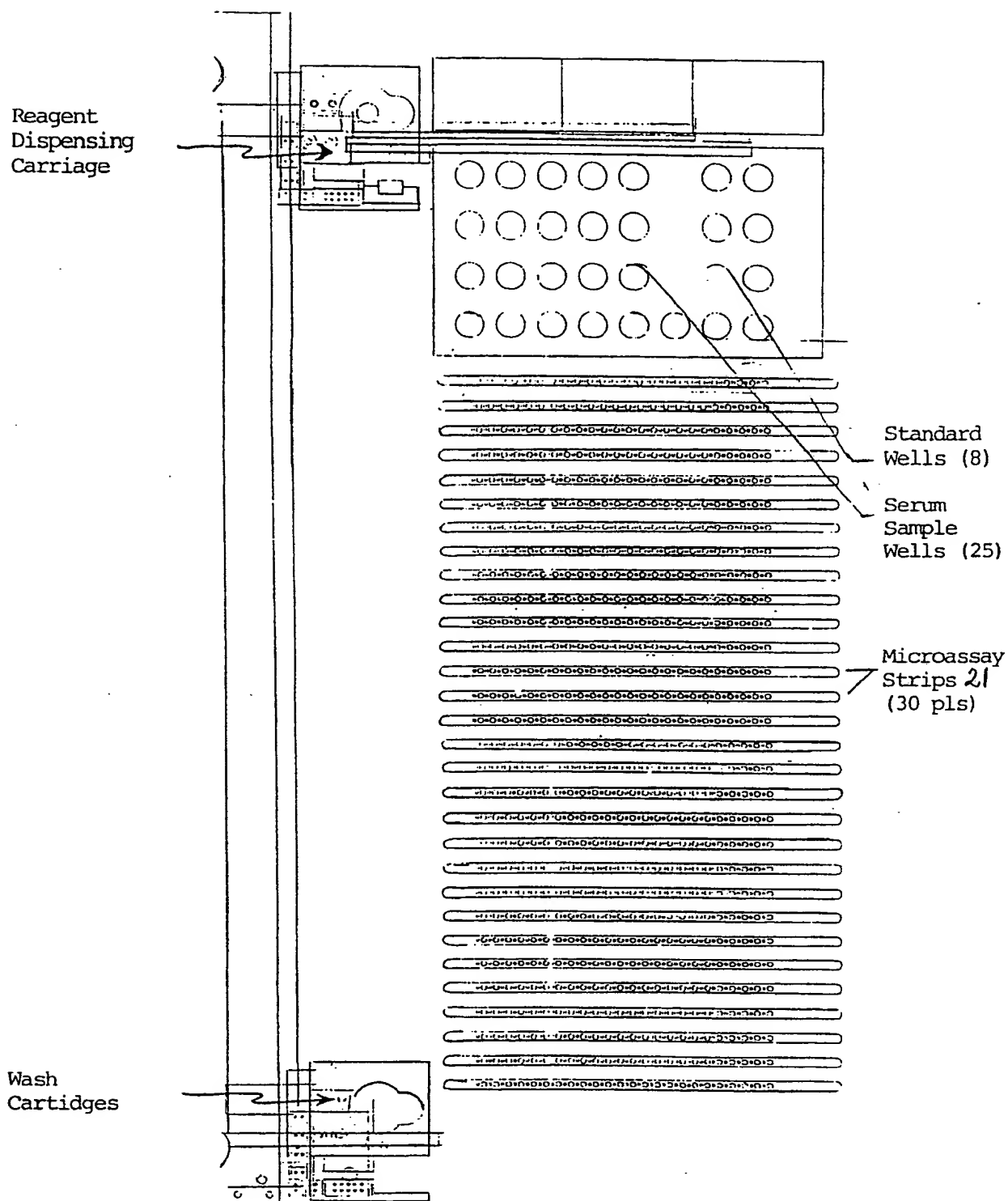


Fig. 2



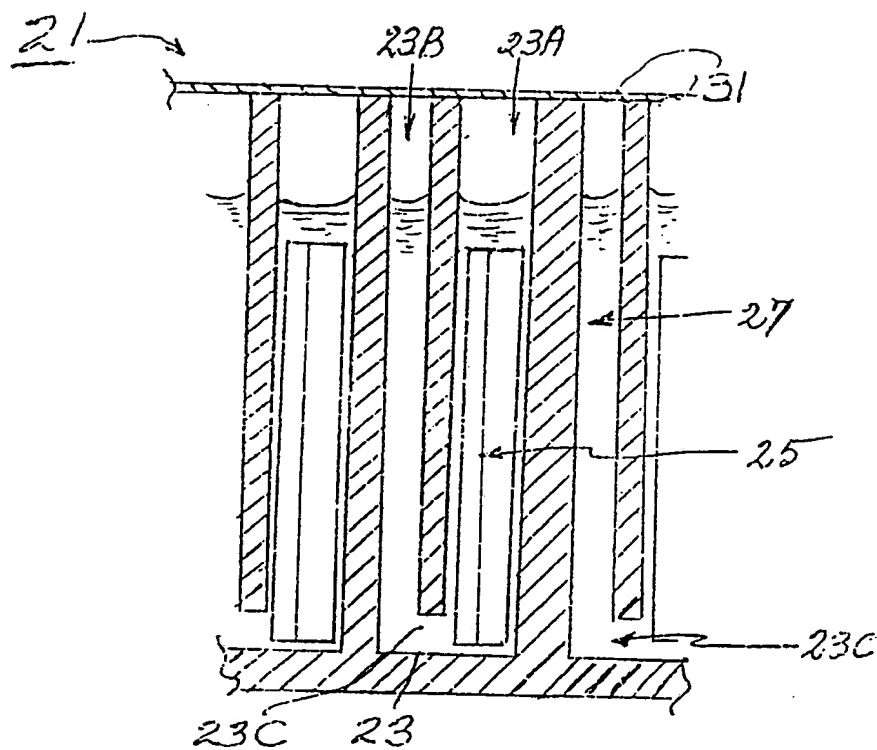


Fig. 3

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/03589

| <b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup><br>According to International Patent Classification (IPC) or to both National Classification and IPC<br>IPC(5): GOIN 21/03, 31/20, 33/53, 33/543, 33/545, 33/552<br>USCL.: 435/7.92, 7.93, 7.94, 7.95; 436/518, 527, 531; 422/58  |  |                                     |  |  |                                     |   |   |      |   |  |      |   |   |      |
|---|--|-------------------------------------|--|--|-------------------------------------|---|---|------|---|--|------|---|---|------|
| <b>II. FIELDS SEARCHED</b><br><div style="text-align: center; font-size: small;">Minimum Documentation Searched <sup>7</sup></div> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%; text-align: left; padding: 5px;">Classification System</th> <th style="text-align: left; padding: 5px;">Classification Symbols</th> </tr> <tr> <td style="padding: 5px; vertical-align: top;">USCL.:</td> <td style="padding: 5px;">435/7.92, 7.93, 7.94, 7.95, 805, 970, 973;<br/>436/518, 527, 531, 809;<br/>422/58</td> </tr> </table> <div style="text-align: center; font-size: x-small;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup></div>   |  |                                     | Classification System  | Classification Symbols   | USCL.:                              | 435/7.92, 7.93, 7.94, 7.95, 805, 970, 973;<br>436/518, 527, 531, 809;<br>422/58 |   |      |   |  |      |   |   |      |
| Classification System   | Classification Symbols   |                                     |  |  |                                     |   |   |      |   |  |      |   |   |      |
| USCL.:  | 435/7.92, 7.93, 7.94, 7.95, 805, 970, 973;<br>436/518, 527, 531, 809;<br>422/58  |                                     |  |  |                                     |   |   |      |   |  |      |   |   |      |
| <b>DIALOG AND AUTOMATED PATENT SYSTEM DATABASES</b>   |  |                                     |  |  |                                     |   |   |      |   |  |      |   |   |      |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; text-align: left; padding: 5px;">Category <sup>9</sup></th> <th style="text-align: left; padding: 5px;">Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></th> <th style="width: 10%; text-align: left; padding: 5px;">Relevant to Claim No. <sup>13</sup></th> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">US.A. 4.146.365 (KAY ET AL) 27 MARCH 1979.<br/>Note: abstract: column 3. line 14-column 4. line 7: FIGURE 2.</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-15</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">EP.A. 0.197.729 (KELLY ET AL) 15 October 1986.<br/>Note: page 2. lines 17-25: page 4. lines 7-29: page 5. lines 3-4: page 6: lines 10-27: page 7. line 14-page 8. line 8.</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-15</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">EP.A. 0.154.687 (SAPATINO ET AL.) 18 SEPTEMBER 1985. Note: page 4, line 13-page 5, line 15: page 11, line 18 page 12, line 1; Figures 2 and 2a.</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-15</td> </tr> </table> |  |                                     | Category <sup>9</sup>  | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>   | Relevant to Claim No. <sup>13</sup> | Y   | US.A. 4.146.365 (KAY ET AL) 27 MARCH 1979.<br>Note: abstract: column 3. line 14-column 4. line 7: FIGURE 2. | 1-15 | Y | EP.A. 0.197.729 (KELLY ET AL) 15 October 1986.<br>Note: page 2. lines 17-25: page 4. lines 7-29: page 5. lines 3-4: page 6: lines 10-27: page 7. line 14-page 8. line 8. | 1-15 | Y | EP.A. 0.154.687 (SAPATINO ET AL.) 18 SEPTEMBER 1985. Note: page 4, line 13-page 5, line 15: page 11, line 18 page 12, line 1; Figures 2 and 2a. | 1-15 |
| Category <sup>9</sup>   | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>   | Relevant to Claim No. <sup>13</sup> |  |  |                                     |   |   |      |   |  |      |   |   |      |
| Y   | US.A. 4.146.365 (KAY ET AL) 27 MARCH 1979.<br>Note: abstract: column 3. line 14-column 4. line 7: FIGURE 2.  | 1-15                                |  |  |                                     |   |   |      |   |  |      |   |   |      |
| Y   | EP.A. 0.197.729 (KELLY ET AL) 15 October 1986.<br>Note: page 2. lines 17-25: page 4. lines 7-29: page 5. lines 3-4: page 6: lines 10-27: page 7. line 14-page 8. line 8.                         | 1-15                                |  |  |                                     |   |   |      |   |  |      |   |   |      |
| Y   | EP.A. 0.154.687 (SAPATINO ET AL.) 18 SEPTEMBER 1985. Note: page 4, line 13-page 5, line 15: page 11, line 18 page 12, line 1; Figures 2 and 2a.  | 1-15                                |  |  |                                     |   |   |      |   |  |      |   |   |      |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Δ" document member of the same patent family</p> </div> </div>  |  |                                     |  |  |                                     |   |   |      |   |  |      |   |   |      |
| <b>IV. CERTIFICATION</b> <table style="width: 100%;"> <tr> <td style="width: 50%; vertical-align: top;">           Date of the Actual Completion of the International Search<br/><br/> <b>27 JUNE 1991</b><br/><br/>           International Searching Authority<br/><br/> <b>ISA/US</b> </td> <td style="width: 50%; vertical-align: top;">           Date of Mailing of this International Search Report<br/><br/> <div style="font-size: 1.5em; font-weight: bold;">19 AUG 1991</div><br/>           Signature of Authorized Officer<br/> <br/> <b>CAROL A. SPIEGEL</b> </td> </tr> </table>   |  |                                     | Date of the Actual Completion of the International Search<br><br><b>27 JUNE 1991</b><br><br>International Searching Authority<br><br><b>ISA/US</b> | Date of Mailing of this International Search Report<br><br><div style="font-size: 1.5em; font-weight: bold;">19 AUG 1991</div><br>Signature of Authorized Officer<br><br><b>CAROL A. SPIEGEL</b> |                                     |   |   |      |   |  |      |   |   |      |
| Date of the Actual Completion of the International Search<br><br><b>27 JUNE 1991</b><br><br>International Searching Authority<br><br><b>ISA/US</b>  | Date of Mailing of this International Search Report<br><br><div style="font-size: 1.5em; font-weight: bold;">19 AUG 1991</div><br>Signature of Authorized Officer<br><br><b>CAROL A. SPIEGEL</b> |                                     |  |  |                                     |   |   |      |   |  |      |   |   |      |

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/03589

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) |   |                      |
|--|---|----------------------|
| Category *   | Citation of Document, with indication, where appropriate, of the relevant passages        | Relevant to Claim No |
| Y  | US.A, 4.849,340 (OBERHARDT) 18 July 1989.<br>Note: column 29, line 40-column 31, line 42. | 1-15                 |
| A  | US. A. 4.756,884 (HILLMAN ET AL.) 12 July 1988. Note: Entire document.                    | 1-15                 |
| A  | EP.A, 0.174.247 (GUERIN ET AL) 12 MARCH 1986. Note: Entire document.                      | 1-15                 |

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